the molecular events that take place in the infected liver. Furthermore, it is becoming increasingly apparent that the vertebrate host does not play a passive role in the infection process and that, on the contrary, it actively influences the fate of infection. In this context, the paper by Coppi et al. (2007) represents a crucial piece of this immense jigsaw.

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Clathrin: An Amazing Multifunctional Dreamcoat?

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Internalization of cargo by clathrin-mediated endocytosis has been studied extensively. In this issue of *Cell Host & Microbe*, Cossart and colleagues report that a variety of pathogens induce the recruitment of clathrin and other endocytic proteins to sites of pathogen interaction with the cell surface. This recruitment is followed by actin rearrangements in the host cell necessary for the uptake or stable attachment of the pathogen at the cell surface.

The plasma membrane serves as a barrier that separates the cytoplasm from the extracellular milieu. Some molecules, like ions, can enter the cell via channels in the lipid bilayer, while other molecules, like growth factors, bind to cell surface receptors and are taken up by endocytosis. There are several types of endocytosis, with clathrin-mediated endocytosis being the most thoroughly studied. Classical clathrin-mediated endocytosis involves the assembly of a clathrin lattice, cargo recruitment via adaptors, formation and release of the vesicle into the cytoplasm, and targeting of the rapidly uncoated vesicle to a specific site within the cell. Besides the key player clathrin, a large number of accessory proteins have been identified that are important for endocytosis, including the adaptor protein AP-2 and, in most cells, the large GTPase dynamin. In addition, actin and actin-regulating proteins, like the Arp2/3 complex and N-WASP, localize to endocytic sites (Figure 1A) (Engqvist-Goldstein and Drubin, 2003). Clathrin-mediated endocytosis usually involves formation of relatively small vesicles (about 100 nm).

Recently, clathrin has been implicated in several nonclassical events at the plasma membrane. Falk and colleagues suggested a role for clathrin in the internalization of large double-membrane vesicles at the lateral membranes of neighboring cells that are coupled by GAP junctions (Figure 1B) (Piehl et al., 2007). Reduction of clathrin levels by RNAi resulted in reduced internalization of GAP junctional clusters. The authors also showed that this process is actin dependent. Additionally, several reports demonstrated that large particles (>1 μm), like latex beads and even certain bacteria and viruses, can utilize the clathrin-associated endocytic machinery and the actin cytoskeleton to enter cells (Aggeler and Werb, 1982; Van Nhieu et al., 1996). Each of these events involves formation of structures substantially larger than classical clathrin-coated vesicles, suggesting previously unappreciated diversity in clathrin's roles and in its organization while mediating cellular processes.

The so-called "zippering" bacteria, such as *Listeria monocytogenes* and *Yersinia pseudotuberculosis*, express proteins on their surfaces that directly interact with receptors on the surfaces of host cells, leading to the clathrin- and actin-dependent endocytosis of the bacteria. InlA and InlB of *Listeria* are among the most extensively studied bacterial surface proteins. InlA binds to E-cadherin, a cell adhesion molecule involved in the formation of intercellular junctions and epithelial cell polarization (Mengaud et al., 1996).

InlB is a ligand for the receptor tyrosine kinase Met (Shen et al., 2000). Upon binding to InlB, Met becomes activated, leading to the Cbl (ubiquitin ligase)-mediated mono-ubiquitination and the subsequent endocytosis of the receptor and the receptor-bound pathogen. In addition to recruiting Met and Cbl to sites of pathogen binding on the host cell, internalizing *Listeria* have been shown to recruit the endocytic proteins clathrin, dynamin, and EEA1, and knockdown of these proteins impairs *Listeria's* ability to infect cells (Veiga and Cossart, 2005).

Cossart and colleagues have now performed a more extensive analysis of clathrin's role in interactions of diverse bacterial pathogens with host cells (Veiga et al., 2007). They showed that upon association with human epithelial cells, noninvasive *E. coli* expressing the *Yersinia pseudotuberculosis* protein invasin, a noninvasive strain of *Listeria* expressing InlA, and *Staphylococcus aureus*, cause recruitment of clathrin and dynamin to sites of contact with the host cell (Figure 1C). Furthermore, lowering levels of clathrin, dynamin, Cbl, and Grb2 (an adaptor protein for Cbl) by RNAi or inhibiting the activity of dynamin by dynasore (a noncompetitive inhibitor of dynamin) results in reduced uptake of invasive bacteria into host cells. Clathrin and dynamin recruitment to invading pathogens was also necessary for subsequent actin polymerization around the internalizing pathogens. In addition, the authors performed dual-color, live-cell imaging with fluorescently-tagged *Listeria* and clathrin and found that the recruitment of clathrin was four to six times slower than during endocytosis of a classical ligand like transferrin, consistent with a different mechanism of clathrin recruitment or assembly.

The authors also discovered a role for clathrin in the attachment of enteropathogenic *Escherichia coli* (EPEC) to the host cell (Veiga et al., 2007). These bacteria do not enter the host cell but use a type III secretion system to inject virulence factors into the cell to induce actin rearrangements that lead to the formation of an actin-rich pedestal underneath the bacterium (Figure 1D). Cossart and colleagues now report

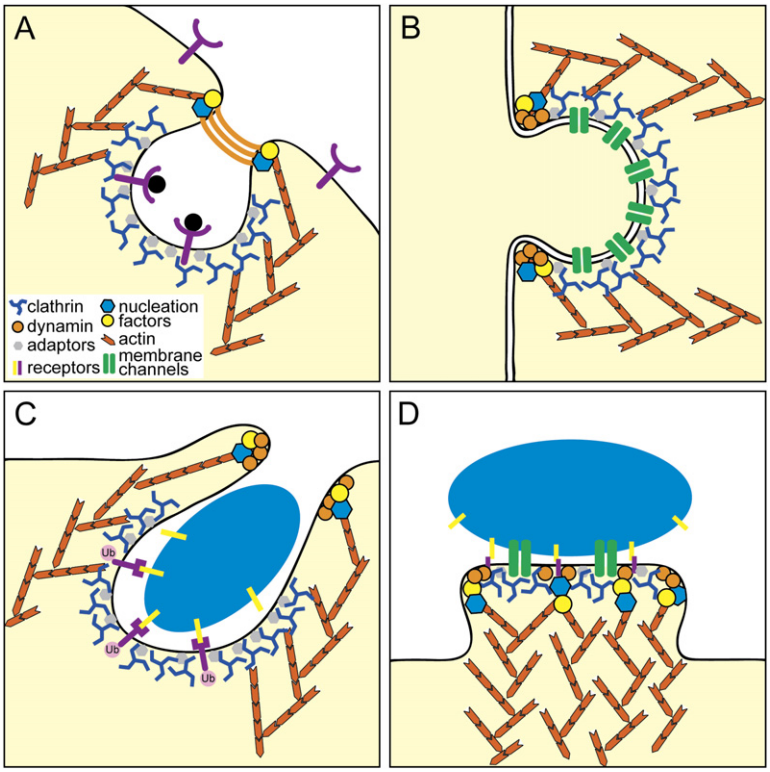

Figure 1. Clathrin, Other Endocytic Proteins, and the Actin Cytoskeleton Are Involved in the Internalization of a Diverse Set of Cargoes and in Pedestal Formation in Mammalian Cells

Classical clathrin-mediated endocytosis (A) involves the recruitment of clathrin, adaptor proteins, dynamin and actin nucleation factors, and the assembly of actin filaments. Ultrastructural studies suggest that dynamin forms a ring-like structure around the neck of the invaginating endocytic vesicle during mammalian clathrin-mediated endocytosis. Whether rings also form during any of the other events described here is currently not known. (B) The uptake of large, double-membrane vesicles containing clusters of GAP junctions is mediated by the endocytic machinery and the actin cytoskeleton. Some bacteria appear to enter (C) or attach to host cells (D) by a similar mechanism, involving the recruitment of clathrin and dynamin, and stimulation of actin filament assembly. The manner in which the proteins are organized is, in each case, speculative. Drawings are not to scale.

that clathrin localizes to the host cell cortex in these actin-rich protrusions and that it is necessary for pedestal formation. Another recent study found that dynamin is also recruited to the cortex of the pedestals (Unsworth et al., 2007). The fact that the bacterium never enters the cell but still induces the recruitment of clathrin to the site of attachment and that this recruitment is necessary for subsequent actin polymerization points toward a regulatory role for clathrin in this process, possibly by recruiting factors that induce actin assembly.

These observations, considered together with recent findings suggesting a regulatory role for clathrin in yeast endocytosis, indicate a greater functional repertoire for clathrin than was previously appreciated. In the yeast studies, Lemmon and colleagues showed that the overexpression of clathrin light chain can overcome the endocytic defects in yeast cells lacking clathrin heavy chain (Newpher et al., 2006). They suggest that clathrin may act as a scaffold to promote the assembly of endocytic proteins like Sla2p (Hip1R in mammals). This conclusion is supported by another study in yeast, wherein the absence of clathrin resulted in a 50% reduction in endocytic events. This defect was not due to impaired vesicle formation or invagination, but rather to a decrease in the number of productive endocytic sites. Therefore, in yeast, clathrin may function as a platform to assemble and/or recruit other coat

proteins (Kaksonen et al., 2005). Since clathrin-mediated endocytosis in yeast and more complex eukaryotic cells share many features, it is now important to investigate whether clathrin in the more complex eukaryotic cells may play analogous roles in the initiation of coat assembly and as a recruitment factor for proteins involved in actin-assembly, both in the uptake of endocytic vesicles and in the internalization of pathogens. It will also be crucial to determine, by electron microscopy, the ultrastructural characteristics of the clathrin coats associated with pathogenic bacteria and yeast endocytic sites, and to identify the proteins that link the clathrin coat to the actin cytoskeleton at the host-pathogen interface.

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